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1 **Complete Sequence of the FII Plasmid p42-2, encoding *bla*<sub>CTX-M-55</sub>, *oqxAB*,**  
2 ***fosA3* and *floR* from *Escherichia coli***

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14 Running title: Complete Sequence of the FII Plasmid p42-2

15

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26 **Abstract**

27 We sequenced a novel conjugative multidrug resistance IncF plasmid, p42-2, isolated from an  
28 *Escherichia coli* strain 42-2 previously identified in China. p42-2 is 106,886 bp in size,  
29 composed of a typical IncFII-type backbone (~54 kb) and one distinct acquired DNA region  
30 spanning ~53 kb, harboring 12 antibiotic resistance genes (*bla*<sub>CTX-M-55</sub>, *oqx*A, *oqx*B, *fos*A3,  
31 *flo*R, *tet*A(A), *tet*A (R), *str*A, *str*B, *sul*2, *aph*(3')-II and *Δbla*<sub>TEM-1</sub>). The spread of these multi-  
32 drug-resistance determinants on the same plasmid is of great concern and because of co-  
33 resistance to antibiotics from different classes is therapeutically challenging.

34

35 **Keywords:** FII Plasmid, multidrug resistance, complete sequencing, mobile genetic elements  
36 (MGEs), *Escherichia coli*

37

Widespread antibiotic resistance poses an enormous threat for human and animal health worldwide (1). This problem has been exacerbated in recent years with the emergence of multidrug-resistant (MDR) plasmids conferring resistance to most classes of antimicrobials (2). Plasmids of the incompatibility F group (IncF), representing one of the most frequently encountered plasmid types (3), have frequently been associated with MDR phenotypes including extended-spectrum  $\beta$ -lactamases (ESBLs) and plasmid-mediated quinolone (PMQR) genes (4, 5). In our previous study, IncF plasmids recovered from *Escherichia coli* of food-producing and companion animals were investigated and most of them carried numerous resistance determinants, such as *bla*<sub>CTX-M</sub>, *rmtB*, *oqxAB*, and *floR* (4, 6). The spread of these multi-resistance plasmids has prompted worldwide concern because of co-resistance to multiple antimicrobial agents that will facilitate the survival of bacteria under the selective pressure of antibiotics. Herein, we analysed a common subtype IncF plasmid, F33: A-: B- (FII, FIA, FIB; FAB formula), this plasmid, designated p42-2, contains 12 different resistance genes, was fully sequenced and the data compared with other IncF plasmids.

*E. coli* 42-2 was recovered from the faeces of a healthy duck in Guangzhou, China. Conjugation was performed by mixing *E. coli* 42-2 and *E. coli* C600 in a liquid medium and isolating for *E. coli* C600 (p42-2) by selecting on MacConkey agar containing streptomycin (1000 mg/L) and cefotaxime (2 mg/L) as previously described (4, 6). p42-2 was extracted from the *E. coli* C600 transconjugant using a commercial kit (Qiagen Midi kit, Qiagen, Germany). Sequencing of p42-2 was carried out on an Illumina genome analyzer IIx with a 500bp paired-end library (approximately 100M available reads, 935-fold genome coverage) and 2000bp paired-end library (approximately 337M available reads, 3150-fold genome coverage), respectively. These raw data were assembled by SOAPdenovo (7). Gene prediction and annotation were performed using RAST tools (8). The sequence comparison

63 and map generation were performed using BLAST (<http://blast.ncbi.nlm.nih.gov>) and Easyfig  
64 version 2.1 (9).

65

66 Plasmid p42-2 confers resistance to ampicillin, chloramphenicol, kanamycin, streptomycin,  
67 sulfonamides/trimethoprim, tetracycline, olaquinox, fosfomycin, cephalosporin and  
68 florenicol. Because of its broad resistance spectrum, p42-2 was sequenced and fully  
69 annotated. p42-2 contains 106,886 bp with 141 predicted open reading frames (ORFs) and is  
70 composed of a typical IncFII-type backbone (~54 kb), encoding functions for IncF plasmid  
71 replication, horizontal transfer, maintenance and stability functions. The remaining ~53 kb is  
72 an acquisition region carrying various resistance genes and mobile genetic elements (MGEs).  
73 The whole sequence of p42-2 is organized similarly to other IncF plasmids, including  
74 pHNFP460-1 (GeneBank accession number KJ020575, mainland China) (Fig.1), and  
75 pHN7A8 (GeneBank accession number JN232517, mainland China) (10), with a 61%~63%  
76 coverage and an overall nucleotide identity of 99%. Sequencing analysis confirm that p42-2  
77 is a chimera plasmid made up of basic an IncF plasmid backbone with an additional ~53 kb  
78 mobile region.

79

80 The p42-2 backbone (replication and transfer regions) is highly conserved with other known  
81 IncF plasmids, such as pHNFP460-1, pHN7A8 and pHK23a (GeneBank accession number  
82 JQ432559, Hongkong) (Fig. S1). p42-2 contains one replication region (*copB*, *repA3*, *repA1*  
83 and *repA4*), belonging to Inc group F, with plasmid replicon type, F33: A-: B-, according to  
84 FAB (FII, FIA, FIB) criteria (11). The p42-2 replication region was flanked by plasmid  
85 functional modules (*pemK/pemI* and *snrB*), which contributes to plasmid maintenance in the  
86 bacterial population (12). This region is identical to that in other IncFII plasmids  
87 pHNFP460-1 and pHN7A8 (Fig.S1). The transfer region of p42-2 comprises of 26 *tra* genes

88 and 6 *trb* genes (*traM*, *traJ*, *traY*, *traA*, *traL*, *traE*, *traK*, *traB*, *traP*, *traD*, *traG*, *traV*, *traR*,  
89 *traC*, *trbI*, *traW*, *traU*, *trbC*, *traN*, *trbE*, *traF*, *traQ*, *trbB*, *trbJ*, *trbF*, *traH*, *traG*, *traS*, *traT*,  
90 *traD*, *traI*, *traX*). The gene order of this region is totally conserved in pHNFP460-1 and  
91 pHN7A8 (Fig.S1).

92  
93 The ~53 kb variable region (position 3996 to 56766) comprises 12 resistance determinants,  
94 complete or truncated insertion sequences and transposons, as well as many genes of  
95 unknown function and was located downstream to the replication region. In comparison with  
96 pHNFP460-1 and pHN7A8, p42-2 is composed of a larger multi-resistance region (MRR),  
97 which is flanked by seven different oriented IS26 elements into five succinct regions  
98 harboring antibiotic resistance genes (Fig.1): The first region comprises six antibiotic  
99 resistance genes (*floR*, *tetA(A)*, *tetA(R)*, *strA*, *strB* and *sul2*), conferring resistance to  
100 florenicol, tetracycline, streptomycin and sulfonamide. This region has highest identity  
101 (99.9%) with part of pCFSA007428.01 (GeneBank accession number CP009414, USA). p42-  
102 2 possesses an additional sequence (~20 kb) in the second region, with many genes of  
103 putative functions, such as plasmid maintenance and stability determinants (*stbD/stbE*) (13),  
104 and genes associated with virulence factors (*vagC/vagD*). Interestingly, partial replication  
105 genes (*bis-repX-pir*) of IncX plasmid also have been found in this region, suggesting that  
106 only part of an IncX plasmid has been captured or acquired of IncX followed by a partial  
107 deletion event. The third region possesses two multi-drug efflux pumps *oqxA* and *oqxB*,  
108 which are active against fluoroquinolones. IS26-like sequences flank this locus (IS26-*oqxA*-  
109 *oqxB*-IS26) which is closely related to pACN001-A (GeneBank accession number  
110 KC853434, China) and pOLA52 (GenBank accession number EU370913, Denmark) (14).  
111 There are an additional four resistance determinants, *tetA(R)*, *fosA3*, *Abla*<sub>TEM-1</sub> and *bla*<sub>CTX-M</sub>.  
112 55, combined by three IS26 elements. This region, IS26-*tetA(R)*-*fosA3*-IS26-*Abla*<sub>TEM-1</sub>-*orf477*-



113 *bla*<sub>CTX-M-55</sub>-*ISEcp1*-IS26, was identical to plasmids pHNFP460-1 and pHP588 (GenBank  
114 accession number AB778291, Japan). p42-2 MRR is flanked by fragments of IS1 with the  
115 same IS26/IS1 backbone as seen in pHN7A8 (Fig.1) and was shown to be combined with a  
116 diverse range of MGEs (IS5075, IS50, ISCR2, IS26, IS1294, *ISEcp1* and Tn21). These  
117 MGEs are important in horizontal gene transfer and may provide a favorable genetic  
118 environment for plasmid plasticity. For instance, a total of seven IS26 copies are located on  
119 p42-2 (Fig.1), each associated with different antibiotic resistance genes such as *bla*<sub>CTX-M-55</sub>,  
120 *oqxAB* and *fosA3*. This observation proves further evidence that IS26 plays an important role  
121 in the dissemination and evolution of IncF antibiotic resistance plasmids by creating regions  
122 containing multiple antibiotic resistance genes through stepwise integration and/or  
123 recombination events mediated by IS26 (15,16). Thus, the variable region of p42-2, encoding  
124 12 antibiotic resistance determinants, may have arisen from MRR acquisition by the actions  
125 of MGEs from multiple plasmid sources, with an IncF plasmid backbone.

126

127 In conclusion, p42-2 is a chimera plasmid made up of basic an IncF plasmid backbone with a  
128 large multi-resistance region. This region appears to have evolved through the integration of  
129 multiple resistance determinants from different sources by the action of MGEs and  
130 recombination. The association of these important antibiotic resistance genes (such as *bla*<sub>CTX-</sub>  
131 *M-55*, *oqxAB*, *fosA3* and *floR*) on the same plasmid is therapeutically challenging due to co-  
132 selection by various drugs and may confer a selection advantage antibiotic resistant *E. coli*  
133 clones.

134

#### 135 Nucleotide sequence accession number

136 The annotated sequence of plasmid p42-2 from strain 42-2 has been submitted to GenBank  
137 accession number KT990220.

138

139

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196

197 **Figure legend**

198 Fig.1 Comparison of p42-2 MRR with other plasmids containing the same resistance  
199 modules. The extents and directions of antibiotic resistance (dark arrows) and other selected  
200 genes (grey arrows) are indicated by labelled arrows (HPs, hypothetical proteins, are  
201 indicated by black boxes). ISs are shown as white boxes labelled with their number/name and  
202  $\Delta$  represents a truncated gene. The plasmid backbone is indicated by dark lines. Light-grey  
203 shading and oblique dotted lines between two regions indicate these regions with homology.  
204 Diagrams are drawn from sequences available under the following GenBank accession  
205 numbers: p42-2, KT990220; pCFSAN007428\_01; CP009414, pACN001-A, KC853434;  
206 pHNFP460-1, KJ020575 and pHN7A8, JN232517.

